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December 5, 2003

E-MAIL AND TELECOPY

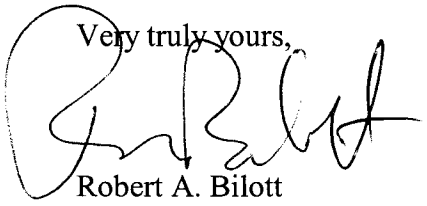
Public Information and Records Integrity Branch (PIRIB)
Office of Pesticide Programs (OPP)
Environmental Protection Agency
1200 Pennsylvania Avenue N.W.
Washington, DC 20460-0001
ATTENTION: Docket No. OPP-2003-0338

Re: Public Comment For USEPA Docket No. OPP-2003-0338: Public Comment

To Docket:

Attached for consideration in connection with USEPA's proposed policy on liver effects from exposure to chemicals alleged to be peroxisome proliferators is a copy of a paper addressing issues raised with respect to adverse liver effects reported among employees exposed to PFOA.

Very truly yours,



Robert A. Bilott

RAB/mdm
Attachment

W0095357.1

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November 7, 2003

TELECOPY AND FEDERAL EXPRESS

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Re: Additional Information Relating To Validity Of Conclusions In 3M Reports Regarding Liver Effects Among PFOA-Exposed Workers

Ladies and Gentlemen:

Among the data submitted to the United States Environmental Protection Agency ("USEPA") in connection with its current evaluation of human health risks associated with exposure to PFOA are two papers authored by Messrs. Olsen, Burlew, Burris, and Mandel in 2001 and 2003 in which the authors conclude that there was no liver toxicity found among PFOA-exposed workers. The 2001 report was referenced and discussed on Pages 32-34 of USEPA's November 4, 2002, Revised Draft Hazard Assessment of Perfluorooctanoic Acid and Its Salts and on pages 23-24 of USEPA's April 10, 2003, Preliminary Risk Assessment Of The Developmental Toxicity Associated With Exposure To Perfluorooctanoic Acid And Its Salts.

The study also is included the public administrative record for PFOA matters at AR-226-1047. The 2003 article referencing the 2001 data appears in Volume 45 of the Journal of Occupational Environmental Medicine at Pages 260-270.

As indicated in the attached report from Tetra Tech, Inc., the conclusions of Olsen, *et al.* with respect to liver toxicity among PFOA-exposed workers are not supported by proper statistical analyses. To the contrary, the data actually reported by the authors support "the unavoidable conclusion that liver toxicity exists in these workers." Given the importance of these data indicating liver toxicity among PFOA-exposed workers, we request that USEPA consider the attached analysis in connection with finalization of its Hazard and Risk Assessments for PFOA and that USEPA take appropriate action to ensure that the correct liver toxicity data are accurately reported. We also request that this letter and attached report be included in AR-226, OPPT-2003-012, and in the IRIS database for PFOA and PFOS.

Very truly yours,

Robert A. Bilott

RAB/mdm
Attachment
cc: R. Edison Hill, Esq. (w/ attachment)
Larry A. Winter, Esq. (w/ attachment)
David G. Gray, Ph.D. (w/ attachment)
James Dahlgren, M.D. (w/attachment)

LIVER TOXICITY

IN

FLUOROCHEMICAL WORKERS

November 2003

Prepared by

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Liver Toxicity in Fluorochemical Workers

An analysis of medical surveillance data on employees exposed to perfluorooctanoate (PFOA) and perfluoro octane sulfonyl (POFS)-based chemicals in the workplace has been published by 3M Company (Olsen et al. 2001). The study is in the U.S. Environmental Protection Agency (EPA) PFOA administrative record as AR226-1047.

POFS-based chemicals may transform, to an undetermined degree, to perfluorooctanesulfonate (PFOS) in the body. The study reports that the more heavily exposed workers, as indicated by elevated PFOA and PFOS serum levels, have higher serum liver enzyme concentrations, suggesting liver damage. The study also performs a multivariate analysis that includes lifestyle and demographic factors that are known to be associated with elevated liver enzyme levels and concludes that it is these factors that are responsible for the indications of liver damage rather than PFOA/PFOS exposure. Unfortunately, Olsen et al. (2001) included independent variables in the analysis that are correlated and, therefore, are not truly independent. Thus, the multivariate analysis violates the assumptions of the statistical method used and, as will be demonstrated herein, the analysis cannot be used to support the conclusion that elevated liver enzyme levels in these workers are attributable to factors other than PFOA/PFOS exposure.

In this cross-sectional study, serum PFOA, PFOS, and total organic fluoride (TOF) levels were measured as indicators of exposure to fluorinated chemicals. The study reports the results of testing for clinical chemistry, thyroid hormone, hematology, and urinalysis parameters for male and female employees working production and nonproduction jobs in the 3M Antwerp and Decatur fluorochemical plants. Since PFOA and PFOS are known liver toxins, measurements of serum concentration for four liver enzymes plus total and indirect bilirubin measurements, were measured as indicators of liver damage. Data on lifestyle and demographic parameters, commonly associated with liver and kidney effects, were also included for each worker, e.g. alcoholic drinks per day, a known risk factor for liver disease. The data were stratified by plant, sex, and production versus nonproduction jobs. These three category variables are correlated with PFOA/PFOS serum levels since PFOA/PFOS exposure is higher in the Decatur versus the

Antwerp plant, in men versus women, and in production versus nonproduction jobs. These categories are used in the study to separate workers according to the magnitude of their exposure and will be referred to as "exposure categories" in this report.

Univariate Analysis – Comparisons Across Exposure Groups

The Olsen et al. (2001) study first presents several univariate analyses directly comparing data across the exposure categories described above. The data show that workers have high liver enzyme levels if they work in the Decatur versus the Antwerp plant, if they are production versus nonproduction workers, and if they are men rather than women. In each case, the group with higher PFOA/PFOS exposure has higher levels of each liver enzyme suggesting liver damage associated with PFOA/PFOS exposure. Statistical testing of the data was done only between plants with the Decatur workers significantly higher than the less heavily exposed Antwerp workers for liver enzymes in men and for three of four enzymes in women. Since the individual data were not provided in the study report, statistical testing of data across sex and job type cannot be done for the present report.

Significant differences between these exposure groups were also observed for variables related to thyroid function. For example, the highest value for TSH, a indicator of possible thyroid impairment, is in Decatur male production workers, the most heavily exposed group.

There were other notable differences between groups including those relating to lipid metabolism and several lifestyle and demographic parameters. For example, employees of the Antwerp plant consumed significantly more alcohol than those in the Decatur plant (1.1 versus 0.1 and 0.5 versus 0.1 drinks/day) for male production workers and female workers, respectively. Thus, alcohol intake runs counter to the usual association with liver damage, indicating the observed differences in liver enzymes between plants could not be explained by this lifestyle factor.

The data presented in Olsen et al. (2001) consisting of direct comparisons across exposure groups indicates the likely presence of liver and thyroid effects associated with PFOA/PFOS exposure in these workers.

Univariate Analysis – Comparison by Quartiles

An additional univariate analysis was conducted by separating the data based on serum PFOA/PFOS quartiles. The quartiles were defined based on PFOS levels, but that designation effectively separates the data by serum PFOA level as well since serum PFOA and PFOS levels are highly correlated in these workers. The quartile PFOA, PFOS, TOF, and ALT data for Decatur male production workers, the most heavily exposed group, are shown in Table 1 below. For these workers, quartile 4, the most heavily exposed quartile, is significantly elevated relative to the other three quartiles for the liver enzyme ALT. Other than the three exposure parameters (PFOA, PFOS, and TOF), and the liver enzyme ALT, no other measured variable was significantly different between any of the quartiles. Moreover, inspection of all the study data including clinical chemistry, thyroid, hematology, and demographic/lifestyle data provides no hint of any differences between quartiles that could account for an elevated liver enzyme value other than the differences in the exposure parameters—PFOA, PFOS, and TOF serum levels.

Table 1
Decatur Male Production Workers
Mean Serum PFOA/PFOS/TOF Concentration (ppm) and Serum ALT

	Quartile 1 (N=40)	Quartile 2 (N=40)	Quartile 3 (N=41)	Quartile 4 (N=40)
PFOA	1.24[3,4]	1.82[4]	2.42[1,4]	3.88[1,2,3]
PFOS	0.55[2,3,4]	1.01[1,3,4]	1.74[1,2,4]	3.22[1,2,3]
TOF	1.34[2,3,4]	2.20[1,3,4]	3.43[1,2,4]	5.75[1,2,3]
ALT[5]	33[4]	32[4]	33[4]	44[1,2,3]

[1]Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 1st Quartile
[2]Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 2nd Quartile
[3]Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 3rd Quartile
[4]Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 4th Quartile
[5]Concentration unit is IU/L

Quartile analysis for the groups exposed to lower PFOA and PFOS levels, such as the Antwerp plant workers, nonproduction workers, and female workers, did not produce statistically significant indications of a dose response for liver effects, possibly because splitting the worker groups into quartiles reduced the number of subjects per group by four, making the statistical detection of group differences more difficult.

When all the workers from both plants were included in a quartile analysis, the indications of liver toxicity in the most heavily exposed quartile persisted with an additional liver enzyme, alkaline phosphatase, significantly elevated in the highest two quartiles. The data are shown in Table 2 below. Since the number of subjects in each quartile is considerably larger, the statistical power of the analysis is improved. Alcohol consumption was significantly lower in quartile 3 and 4 compared to quartile 1 and was again not associated with liver toxicity.

Table 2
Decatur and Antwerp Male Production and Nonproduction Workers
Mean Serum PFOA/PFOS/TOF Concentration (ppm) and Serum ALT/Alk Phos

	Quartile 1 (N=105)	Quartile 2 (N=105)	Quartile 3 (N=106)	Quartile 4 (N=105)
PFOA	0.54[2,3,4]	1.21[1,3,4]	1.45[1,4]	2.70[1,2,3]
PFOS	0.27[2,3,4]	0.60[1,4]	1.19[1,2,4]	2.69[1,2,3]
TOF	0.62[2,3,4]	1.40[1,3,4]	2.12[1,2,4]	4.41[1,2,3]
ALT[5]	26[4]	28	28	33[1]
Alk Phos[5]	61[3,4]	67	69[1]	70[1]

[1]Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 1st Quartile
[2]Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 2nd Quartile
[3]Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 3rd Quartile
[4]Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 4th Quartile
[5]Concentration unit is IU/L

Univariate analysis—Values Exceeding Normal Range by PFOA/PFOS Quartiles

An additional quartile analysis on all workers was performed reporting the number of workers in each exposure quartile with serum liver enzyme concentrations above the reference range

(normal value range). This value is an indicator of how many workers are manifesting clinically recognizable liver damage as opposed to those who may be experiencing more subtle effects. As with the previous univariate analyses the results were stratified by exposure category, i.e. by plant, by sex, and by production versus nonproduction job (Table 3).

For male production workers in the Decatur plant, the most highly exposed Q4 group, a greater number of workers had elevated serum levels than the other three quartiles for AST, ALT, GGT, and total liver panel. In the less heavily exposed Antwerp plant, Q4 male production workers were elevated relative to the other quartiles only for GGT. Looking across all four liver enzymes in Table 3, the number of workers exceeding reference range values is higher for men than women, for production versus nonproduction jobs, and for workers in the Decatur plant versus the Antwerp plant.

Table 3
Number of Workers Exceeding Normal Values for Hepatic Clinical Chemistry
by PFOA/PFOS Quartile and Exposure Category

	Alkaline Phosphatase				AST				ALT				GGT				Total Liver Panel*			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
	Decatur																			
Males/Production	1	2	2	1	0	1	0	4	3	5	3	11	4	3	2	6	7	8	7	14
Males/Nonproduction	0	0	0	0	0	2	0	0	1	1	2	0	0	3	0	1	1	5	2	1
Females/Prod+Nonprod	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0	1	0	2	0
	Antwerp																			
Males/Prod	0	0	0	0	1	0	0	0	1	0	0	0	1	1	2	4	3	5	4	5
Males/Nonproduction	0	0	0	0	0	0	0	1	0	1	0	0	1	1	2	1	2	2	3	1
Females/Prod+Nonprod	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

*Number exceeding normal range for any enzyme or total or direct bilirubin.

In summary, the quartile analysis presented in the 3M report clearly indicates a dose response for serum liver enzyme concentrations across serum PFOA/PFOS quartiles and between the exposure categories (sex, job type, and plant location) that are surrogates for exposure to PFOA/PFOS. Furthermore, the liver enzymes levels for a number of study subjects exceeded the upper bound of the normal range, indicating clinical liver toxicity. Even considering only the values exceeding the normal range, a dose response for the across exposure variables and categories is apparent. The statistical analyses presented by Olsen et al. (2001) and an inspection of demographic and lifestyle variables that might explain the elevated liver enzyme levels fails to provide any potential cause for the observed dose response other than exposure to PFOA/PFOS. Therefore, based on these univariate analyses one would conclude that exposure to PFOA/PFOS is likely to be causally associated with elevated serum liver enzyme levels resulting from liver toxicity in these worker populations.

Multivariate analysis

The Olsen et al. (2001) study dismisses the findings of the univariate analysis based on the results of a multivariate study that purports to demonstrate that the univariate results are due to "confounding factors" and not to PFOA/PFOS exposure. Olsen et al. (2001) states the following.

> "However, after adjusting to the employees' individual liver function values by potential confounding factors including age, BMI, number of alcoholic drinks per day, cigarettes per day and serum triglyceride values, we found no association between liver function values and PFOS or PFOA. We therefore suspect the univariate associations were influenced by known confounders of liver function analyses."

In a subsequently published journal paper reporting on the same data reported in the Olsen et al. (2001), Olsen et al. (2003) also dismisses the elevated liver enzymes in a similar fashion. "Adjusting for potential confounding factors, there were no substantial associations between

hepatic enzymes and the employees' serum PFOS concentrations." The more recent paper does not show the details of the multivariate analysis on which it bases these conclusions.

Olsen et al. (2001) reported using multivariate regression to test for associations between various markers of liver toxicity, such as liver enzyme levels in serum, and various potential causal factors. The potential causal factors included PFOA, PFOS, or TOF serum level, and various lifestyle and demographic factors. The markers of liver toxicity, such as an individual serum liver enzyme, were the dependent variables and the potential causal factors were the independent variables in the analysis. The primary problem with this analysis is that the independent variables included in the regression equations are not independent. Each of the multivariate regression analyses that are presented in Olsen et al. (2001) contains independent variables that are highly correlated and not independent.

The multivariate analysis reported in Olsen et al. (2001) is a linear model fit by the "least square" method. The least square method makes strong assumptions about the structure of the data under study. When these assumptions are violated, the least squares method may completely misrepresent the data and the conclusion suggested by the results may not be correct. Regression diagnostics can be used that reveal these violations of the assumptions. Olsen et al. (2001) did not report the use of regression diagnostics and their analysis obviously violated many of the requirements of the statistical procedure employed.

A well-known requirement in conducting a multivariate linear regression is that the independent variables be truly independent of one another. This means that there must be no intercorrelation between any two explanatory variables (the technical term for this is collinearity). If this intercorrelation exists, computations are inaccurate, coefficients are unstable and their standard error is large. When there is a strong linear relation between predictors in a regression analysis, the precision of the estimated regression coefficients declines and conclusions are inaccurate (Fox and Monett 1997). If this occurs, as in this case, the regression analysis is faulty and cannot be used to draw conclusions.

Unfortunately, the Olsen et al. (2001) study apparently paid little attention to the requirement that independent variables be independent. For example, the study used the following nine independent variables, many of which would be expected to be highly correlated, in the regression analysis for serum ALT, one of the serum liver enzymes reflecting liver damage.

PFOA
Production/nonproduction job
Antwerp/Decatur plant
Age
BMI
Cigarettes/day
Alcoholic drinks/day
Years worked
Triglycerides

For this regression on ALT, the primary purpose of the analysis is to determine whether the variability in serum PFOA levels explains some of the variability in serum ALT levels and may, therefore, be causally related to it. This is done by testing the coefficient of serum PFOA in the regression equation to determine if it is significantly different from zero. The P value for the coefficient of the serum PFOA obtained by Olsen et al. (2001) was 0.13. Since a P value of less than 0.05 is generally required for such a variable to be considered as significantly different for zero, the analysis was judged to have not yielded a significant association between serum PFOA and ALT levels. However, that conclusion is flawed.

The inclusion of another variable in the analysis that is correlated with serum PFOA violates the assumptions of the analysis and invalidates any conclusion regarding the significance of an association between PFOA and ALT. The variable that is correlated with serum PFOA is the plant location variable (Decatur or Antwerp). The PFOA serum levels in Decatur workers are roughly twice that of Antwerp workers because the exposure is higher in the Decatur plant. Since the Antwerp/Decatur variable was intercorrelated with the PFOA level, the coefficients on

which the Olsen et al. (2001) study based its conclusions can be expected to be unstable, have large standard errors, and be inaccurate. Intercorrelated variables such as the Antwerp/Decatur variable should not have been included in the analysis. This could have been avoided if standard diagnostic methods that are used to run regression models when colinearity may exist should have been used. If these precautions had been taken, the PFOA variable might well have been found to be a predictor of serum ALT.

The same problem exists for the multivariate regression on GGT. If standard methods had been used, PFOA or PFOS may have been judged to explain a significant portion of the variability for this enzyme as well. The problems with the ALT and GGT regressions are the most significant as far as determining whether perfluorinated chemical exposure to the Antwerp and Decatur worker populations have resulted in significant liver toxicity. There are other examples of intercorrelated independent variables in the many multivariate regressions that were done for this study. For example, years worked and age will also be to highly correlated variables

When there are many potential predictors, standard variable selection techniques can be used that reduce the predictors to an optimal subset. They can include interaction terms (e.g. alcoholic drinks/day*age) as predictors and they assess their significance in the model.
Also, if the insignificant predictors are kept in the model along with the significant predictors, this will decrease the model fit i.e. artificially compromise (decrease) the effect of the significant variables in explaining the data and lead to incorrect conclusions. A way to avoid chance findings in regression analysis is to run a "cross validation" check. This is done by splitting the data in half to validate the conclusions made from one half to the other or, in this case, by fitting a model to the data for workers from each plant location separately.

Conclusion

The major finding of both the Olsen et al. (2001) and (2003) studies is that there are no indications in the Decatur and Antwerp 3M worker populations of hepatotoxicity. This finding depends entirely on the results of multivariate regressions to invalidate the positive statistical findings of the univariate analysis described above. Had the standard statistical procedures been

properly used in conducting the regression analysis, the assessment of the results of the univariate analysis likely would not have dismissed the unavoidable conclusion that liver toxicity exists in these workers.

References

Fox John, (1997) Applied Regression, Linear Models, and related Methods, Second Edition, Sage Publications, London , England.

Olsen, GW, Burlew, MM, Burris, JM, Mandel JH. 2001a. A cross-sectional analysis of serum perfluorooctanesulfonate (PFOS) and perfluorooctanate (PFOA) in relation to clinical chemistry, thyroid hormone, hematology and urinalysis results from male and female employee participants of the 2000 Antwerp and Decatur fluorochemical medical surveillance program. 3M Company. USEPA Public Docket AR-226-1047*.

Olsen GW, Burris JM, Burlew MM, Mandel JH. 2003. Epidemiological study of Worker Serum Perfluorooctananesulfonate (PFOS) and Perfluorooctanate (PFOA) Concentrations and Medical Surveillance Examinations. J Occup Environ MED. 45:260-270.